

## AMENDMENTS TO THE SPECIFICATION

**Please amend the paragraph beginning on page 34, line 13 of the specification as follows:**

**Rapid DNA extraction.** Upon reception, the swabs were processed prior to PCR amplification following a rapid DNA extraction procedure described in PCT patent publication WO 03/008636. In brief, 50  $\mu$ L of the swab suspension or 100  $\mu$ L of a diluted mid-log bacterial culture (*E. coli* carrying the IC template DNA into pCR2.1) equivalent to the turbidity of a 0.5 McFarland standard was transferred to a 1.5-mL, screw-capped microtube containing 0.05 g of sterile, acid-washed glass beads. Two sizes of beads, the first ranging from 150 to 212  $\mu$ m and the second from 710 to 1180  $\mu$ m were mixed in a 4:1 ratio. The microtube was vortexed at maximum speed for 5 min on a Genie2GENIE2™ model vortex™ (Fisher Scientific). After a quick centrifuge spin, the microtube was heated for 2 min at 95°C.

**Please amend the paragraph beginning on page 35, line 15 of the specification as follows:**

**PCR amplification.** Real-time PCR amplifications were performed either from purified genomic DNA prepared by using the G-NoneGNOME™ DNA isolation kit (Qbiogen) or directly from vaginal/anal specimens. Amplification reactions were performed in a 25  $\mu$ L reaction mixture containing 50 mM Tris-HCl (pH 9.1), 16 mM ammonium sulfate, 8 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of primer Sag59 and 0.8  $\mu$ M of primer Sag190, 0.2  $\mu$ M of the GBS-specific molecular beacon, 0.4  $\mu$ M of the IC molecular beacon, 200  $\mu$ M each of the four deoxynucleoside triphosphates, 450  $\mu$ g/mL bovine serum albumine, 1.25 unit of KlenTaq1™ DNA polymerase (AB Peptides) combined with TaqStartTAQSTART™ Taq polymerase antibody (BD Biosciences) and the IC template. The IC template used in PCR amplifications was either (i) on a recombinant plasmid into *E. coli* INV $\alpha$ FTM (Invitrogen) cells in the mid-log phase of growth at the equivalent of approximately 0.3 *E. coli* cells per PCR reaction to provide an ICSPAD control or (ii) in the same recombinant plasmid purified and linearized by digestion with *Eco*RI at 100 copies per PCR reaction as previously described to provide an ICAD control (Ke *et al.*, 2000, Clin. Chem. **46**:324-331). Reaction mixtures were subjected to thermal cycling (3 min at 94°C, and then 45 cycles of 5 sec at 95°C for the denaturation step, 14 sec at 56°C for the annealing step, and 5 sec at 72°C for the extension step) using a Smart CyclerSMART CYCLER™ PCR instrument (Cepheid). The GBS-specific and IC-specific

amplification/detection was monitored in real-time by measuring the fluorescence signal at every PCR cycle.

**Please amend the paragraph beginning on page 42, line 24 of the specification as follows:**

**Sample preparation.** The nasal swabs were processed prior to PCR amplification following a rapid DNA extraction procedure described in PCT patent publication WO 03/008636. In brief, 50  $\mu$ L of the swab suspension was transferred to a 1.5-mL, screw-capped microtube containing acid-washed glass beads (see Example 1). Then, approximately 13500 *B. globigii* spores prepared as described in Example 2, were used to spike each nasal specimen. Subsequently, either  $1 \times 10^3$ ,  $1 \times 10^4$ , or no *Staphylococcus aureus* cells in mid-log phase of growth were also added to the nasal specimens in a final volume of 70  $\mu$ L. The microtube was vortexed at maximum speed for 5 min on a Genie2GENIE2™ model vortex (Fisher Scientific). After a quick centrifuge spin, the microtube was heated for 2 min at 95°C.

**Please amend the paragraph beginning on page 43, line 18 of the specification as follows:**

**PCR amplification.** Real-time PCR amplifications were performed directly from nasal specimens. Amplification reactions were performed in a 25  $\mu$ L reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM potassium chloride, 0.1% Triton, 3.45 mM  $MgCl_2$ , 0.4  $\mu$ M of primers XSau325 (5'-GGATCAAACGGCCTGCACA-3'; SEQ ID NO: 5) and mec1V511 (5'-CAAATATTATCTCGTAATTTACCTTGITC-3'; SEQ ID NO: 6) targetting the SCCmec right extremity junction (MREJ) type iv, 0.2  $\mu$ M of primers ABgl158 (5'-CACTTCATTTAGGCGACGATACT-3'; SEQ ID NO: 7) and ABgl345a (5'-TTGTCTGTGAATCGGATCTTTCTC-3'; SEQ ID NO: 8) targetting the *B. globigii* *atpD* gene encoding the ATPase, 0.1  $\mu$ M of the MRSA-specific (5'-FAM-CGTCTTACAACGCAGTAACTACGCACTATCATTACAGC-BHQ-1-3'; SEQ ID NO: 9) TaqMan fluorescent probe, either 0.1  $\mu$ M of the *B. globigii*-specific the TaqMan fluorescent probe (5'-TET-CGTCCCAATGTTACATTACCAACCGGCACTGAAATAGG-BHQ-1-3'; SEQ ID NO: 10) or the fluorescent probe targetting targetting the purified and linearized recombinant plasmid (5'-TET-ATGCCTCTTCACATTGCTCCACCTTTCTGTG-BHQ-1-3'; SEQ ID NO: 11), 200  $\mu$ M each of the four deoxynucleoside triphosphates, 340  $\mu$ g/mL bovine serum albumine, 0.035 U of *Taq* DNA

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polymerase (Promega) combined with ~~TaqStart~~TAQSTART™ Taq polymerase antibody (BD Biosciences), 100 copies of the purified and linearized recombinant plasmid carrying the IC template and 2.5 µL of nasal specimen spiked with bacterial spores as described above. Reaction mixtures were subjected to thermal cycling (3 min at 95°C, and then 48 cycles of 5 sec at 95°C for the denaturation step, 15 sec at 60°C for the annealing step, and 15 sec at 72°C for the extension step) using a ~~Smart-Cycler~~SMART CYCLER™ PCR machine (Cepheid). The MRSA-specific and IC-specific amplifications/detections were monitored in real-time by measuring the fluorescence signal at every PCR cycle.